How Effective is Sharpshooter Control at Limiting Pierce's Disease Spread in California Vineyards?

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ABSTRACT

Pierce's disease management in southern California vineyards hinges on chemical control of populations of the vector, the invasive glassy-winged sharpshooter (*Homalodisca vitripennis*), residing in citrus. Systemic insecticides (imidacloprid) are regularly applied to citrus, which is a preferred plant type for the sharpshooter, to reduce insect abundance before they move into vineyards. These treatment programs have been successful, reducing regional sharpshooter populations to a fraction of what they once were. Grape growers also frequently apply systemic insecticides in vineyards, but the efficacy of these treatments for disease management is not known. Over the last three years we conducted a series of surveys in treated and untreated vineyards in Temecula Valley to determine the relative economic value of within-vineyard chemical control for Pierces disease management. In each of the past three seasons we surveyed 34 vineyards in the Temecula Valley that differ in their use of systemic insecticides, and monitored regularly populations of sharpshooters and beneficial insects. Among the years overall Pierce's disease prevalence was low; averaging approximately 1% based on visual symptoms. Prevalence differed slightly among fields of different treatment categories with the lowest infection rates in those vineyards that were either consistently or intermittently treated with imidacloprid. Based on sticky trap monitoring, consistently or intermittently treated vineyards also had lower catches of sharpshooters than untreated fields, but natural enemy catch did not differ appreciably among the three treatment categories. Finally, tap sampling results showed slightly lower natural enemy abundance in consistently treated sites, but the abundance of non-predatory arthropods was also substantially lower in those sites. Collectively, these results suggest that imidacloprid treatments may reduce slightly disease spread, at least in part due to reductions in vector pressure, but without any clear non-target

effects on natural enemies that may lead to secondary pest outbreaks. However, it may not be critical to treat vineyards every year; at least not as long as regional vector populations continue to be reduced through areawide control programs.

Keywords: *Xylella fastidiosa*, vector-borne pathogen, vector control, transmission efficiency, disease prevalence, disease incidence

INTRODUCTION

Chemical control of insect vectors plays a crucial role in many disease mitigation programs. This is true not only for the management of mosquito-borne diseases of humans, such as malaria and dengue fever [8, 9, 26], but also for limiting disease epidemics in a wide range of agricultural crops [12, 22]. In southern California vineyards chemical control at both the areawide and local scales may affect the severity of Pierce's disease, caused by the pathogenic bacterium *Xylella fastidiosa*, by reducing the density or activity of the primary vector, the invasive glassy-winged sharpshooter (*Homalodisca vitripennis* [7, 14]).

Xylella fastidiosa is endemic to the Americas, and is widespread throughout the western and southeastern U.S. This xylem-limited bacterium is pathogenic to a wide variety of plants, including several important crop, native, ornamental, and weedy species [15, 20]. In the Western U.S. the most economically significant host is grapevine, in which *X. fastidiosa* causes Pierce's disease. Multiplication of the bacterium in vines plugs xylem vessels, which precipitates leaf scorch symptoms and typically kills susceptible vines within a few years [20].

X. fastidiosa can be spread by several species of xylem sap-feeding insects, the most important being the sharpshooter leafhoppers [21]. Historically Pierce's disease prevalence has been moderate, with a pattern that is consistent with primary spread into vineyards from adjacent riparian habitats by the native blue-green sharpshooter (*Graphocephala atropunctata* [19]). However, beginning in the late 1990s severe outbreaks occurred in southern California and the southern San Joaquin Valley that are attributable to the establishment and proliferation of the glassy-winged sharpshooter. This invasive sharpshooter is not inherently more efficient at transmitting the pathogen than are native sharpshooters [1, 10]. Instead its threat as a vector appears to stem from a combination of ability to achieve extremely high densities [3] and promote vine-to-vine (i.e. secondary) disease spread [2].

Citrus trees themselves are not susceptible to the strains of *X. fastidiosa* found in

the U.S. (though strains found in Brazil have caused significant economic losses to their citrus industry – [20]). None-the-less citrus plantings figure prominently in the epidemiology of *Xylella* diseases in California. Many portions of southern California and the southern San Joaquin Valley have vineyards in close proximity to citrus groves [23]. This is important because citrus is a preferred habitat for the glassy-winged sharpshooter at key times of the year, allowing this vector to achieve very high densities [4, 16, 17]. High vector populations then disperse seasonally out of citrus into nearby vineyards, resulting in clear gradients of Pierce's disease prevalence (i.e. proportion of infected plants) as a function of proximity to citrus [18].

Given the importance of citrus in Pierce's disease epidemiology, citrus groves have been the focus of areawide chemical control programs, initiated in the Temecula and Coachella Valleys in the early 2000s and shortly afterward in Kern and Tulare Counties [14, 23]. The southern California programs use targeted application of systemic insecticides, such as imidacloprid, to limit *H. vitripennis* populations residing within citrus. Census data in citrus show substantial year to year variation in sharpshooter abundance that may stem from incomplete application, the use of less effect organically-derived insecticides, or inadequate irrigation to facilitate uptake - which makes the consistent management of sharpshooter populations a challenge [24, 25]. None-the-less trap counts have been, overall, much reduced compared to pre-areawide counts. The effect of chemical control can be seen clearly in early insect surveys which found significantly fewer sharpshooters in treated relative to untreated citrus and in vineyards bordering treated versus untreated groves (R. Redak and N. Toscano, unpublished data). Thus, these areawide control programs have been considered successful in southern California [16, 24, 25], and the swift implementation of an areawide management program in Kern County has been credited with limiting the severity of Pierce's disease outbreaks [23].

Research into imidacloprid uptake by grape also has been initiated, and target concentrations high enough to suppress glassy-winged sharpshooter activity (approx. 10 μg/L of xylem sap) can be achieved and will endure for several weeks in mature vines [6]. This information coupled with the success of areawide programs in citrus appears to have led to relatively widespread adoption by grape growers of imidacloprid application in vineyards to reduce further exposure to *X. fastidiosa*. In Temecula Valley, for example, it is estimated that 70% of vineyards use imidacloprid, at an approximate cost of $150-200 per acre (N. Toscano, personal communication). Yet consistent treatment of

vineyards with systemic insecticides is neither universal, nor have there been any measures of how effective these costly treatments are at reducing Pierce's disease.

We studied the epidemiological significance of chemical control in vineyards, via a multi-year series of field surveys in Temecula Valley. This work addresses gaps in empirically-derived observations regarding the cascading effects of vineyard imidacloprid applications on glassy-winged sharpshooter abundance and, ultimately, Pierce's disease severity. The overall goal of this project was to understand **does within-vineyard sharpshooter chemical control reduce vector pressure and Pierce's disease spread?** As part of this overall objective we have been evaluating the following set of research questions:

1. Do vineyards from different treatment categories (untreated, intermittent treatments, or consistently treated) differ in insecticide concentration?

2. Do imidacloprid applications reduce vector abundance or activity in vineyards?

3. Do treatments reduce disease spread in vineyards?

4. Are treatments disrupting biological control and contributing to secondary pest outbreaks?

MATERIALS AND METHODS

In the summer of 2010 we interviewed several vineyard owners and vineyard managers in the Temecula region to identify vineyards with a range of imidacloprid treatment histories. Of the 88 distinct properties for which we acquired information 66 were treated regularly with imidacloprid, 14 were treated intermittently (one to three years of the last 5), and 8 properties were not treated with imidacloprid for at least the last 4 years. 34 of these properties (8 to 14 replicate fields from each of the three treatment categories) were then used for disease surveys and arthropod monitoring over the next three seasons (Fig. 1).

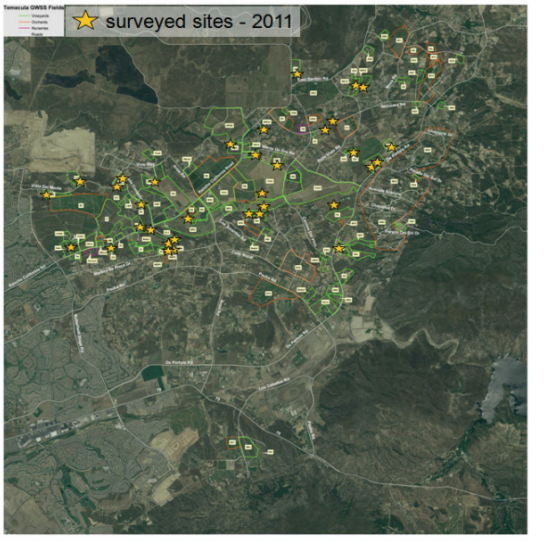

Fig. 1. Location of 34 vineyard sites throughout Temecula Valley (approximately 90 miles southeast of Los Angeles, CA) for use in Pierce's disease and arthropod surveys.

First, to verify that field sites were appropriately categorized based on recent insecticide use history, in late summer we collected leaf samples from 10 vines at each site. For each site 2 vines were sampled in 5 different rows spread throughout the vineyard block. For each vine sampled we collected 2 healthy, fully expanded leaves from mid-cane. These samples were then subjected to an ELISA analysis to calculate imidacloprid concentration, using a slight modification to established methods [6, 7]. Briefly, from each leaf we used a #6 cork borer to punch a disc of leaf tissue, weighed that disc, and then ground it in 1% methanol. After incubation dilutions were made and this material was added to Envirologix Imidacloprid Quantiplate Kit.

In the second and third years of the study we surveyed monthly populations of *H. vitripennis*, the native smoke-tree sharpshooter, *Homalodisca liturata*, and generalist natural enemies. We placed 4-8 yellow sticky-card traps at 0.5-1 m above the vines at each site. The number of traps depended on the area of vines planted. Traps were replaced monthly. For each trap, we counted the number of *H. vitripennis*, *H. liturata*, and four groups of generalist predators: minute pirate bugs (*Orius* spp.), assassin bugs (Reduviidae), big-eyed bugs (*Geocoris* spp.), and spiders (Aranea). These four groups appear to be the most important generalist predators of *H. vitripennis* [11].

In addition to sticky traps, over the summer of 2012 we conducted tap sampling from each of the sites to estimate the abundance of generalist predators and pest insects. At each site, 6 rows approximately evenly spaced throughout the vineyard block were chosen for sampling, avoiding edge rows. Within each row, 5 vines approximately spaced throughout the row were "tap-sampled," avoiding vines at the end of rows, with no neighbors, or which were obviously diseased/dying. Tap sampling was conducted by tapping the vine 40 times to dislodge arthropods from the upper canes, foliage and branches into a shallow beat net. Arthropods collected within a single row were pooled and aspirated into plastic vials and placed on ice during transport. Upon return to the lab, vials were partially filled with 70% ethanol and placed in the freezer until identification.

In the fall of each year we surveyed each vineyard for Pierce's disease prevalence. This time of year was chosen because disease symptoms are strongest late in the year. At each size all vines were visually inspected for obvious leaf scorch-type Pierce's disease symptoms to estimate a percent of vines showing disease. These visual estimates of disease prevalence were adjusted for false positives using plate culturing of bacteria and for false negatives using ELISA. For false positive testing, we collected

petioles from 50 randomly selected vines that were visually categorized as symptomatic for Pierce's disease. We then plate cultured isolates from these petioles for detection of *X. fastidiosa* [13]. To estimate the frequency of false negative, we collected petioles from 100 randomly selected asymptomatic vines found in the vicinity of symptomatic vines. These 100 samples were then subjected to ELISA tests for the presence of *X. fastidiosa*. Estimated false positives and false negatives were used to adjust the disease prevalence based on the visual surveys.

RESULTS AND DISCUSSION

Estimates of imidacloprid concentrations *in planta* match the classes for management practices. All but one of the untreated sites had no detectable imidacloprid in leaf tissue samples. Conversely, both the regularly treated and those treated intermittently (i.e. "mixed" treatment history) had markedly higher insecticide concentrations (Fig. 2A). These results support the classification of most sites into the three treatment categories. Notably, however, several of the mixed treatment sites tended to have equivalent (or even higher) average imidacloprid concentrations to those of consistently treated sites in some years, indicating recent treatments occurred at those sites.

Over the two years of sticky trap monitoring more than 150 sharpshooters were caught in vineyards, the vast majority of which were the invasive glassy-winged sharpshooter. For example, we collected more than 75 sharpshooters, both *H. vitripennis* and *H. liturata,* among all sites between November 2011 and May 2012. In both years, mean sharpshooter catch depended on treatment history grouping. Untreated sites had the highest sharpshooter catches, consistently treated sites were intermediate, and mixed sites had the lowest catches over this period (Fig. 2C). It plausible that regularly treated sites had relatively higher catches than mixed treatment sites because their location exposes them to inherently more sharpshooters (i.e. proximity to citrus); hence encouraging more frequent applications by growers. Regardless, these results overall support the idea that imidacloprid application reduces vector pressure, through mortality or by having anti-feedant effects[5], which may underlie any differences in disease spread among the treatment categories.

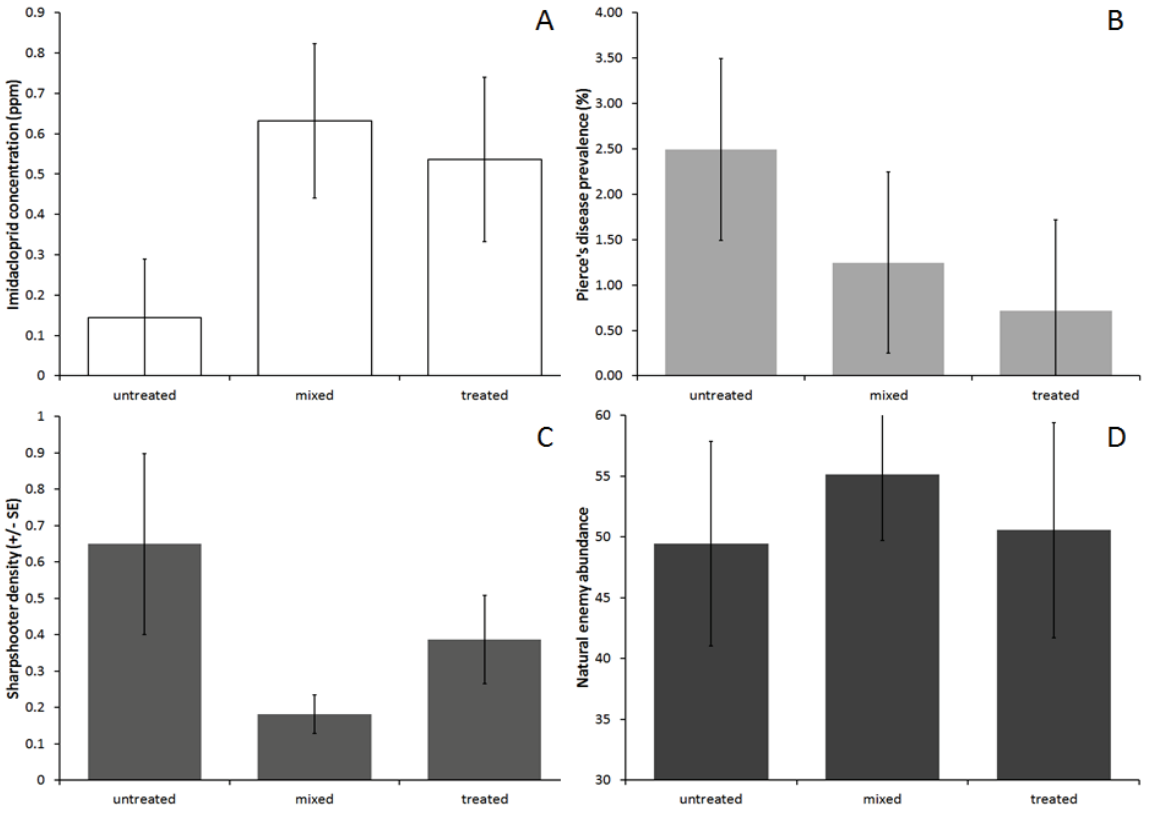

Fig. 2. A) Mean imidacloprid concentration, B) mean estimated Pierce's disease prevalence, C) mean sharpshooter catch on sticky traps, and D) mean natural enemy catch on sticky traps among 34 Temecula vineyards based on treatment category: untreated, intermittently treated (i.e. "mixed") or consistently treated with imidacloprid. Data shown for only 2012.

Estimated Pierce's disease prevalence varied substantially among fields from several sites that had no detectable cases of disease to a single site with on the order of 10% infection. Yet, overall prevalence among the three years of disease surveys was consistently low, with an overall mean of approximately 1.3%. The results suggest that at least part of the variability in prevalence is attributable to systemic insecticide treatment history (Fig. 2B). Specifically, Pierce's disease prevalence tended to be higher in untreated sites, was lowest in the consistently treated sites, and was intermediate in intermittently treated sites. Though not significant, this trend is at least consistent with the expectation of beneficial, albeit only slightly, effects of imidacloprid application on reducing vector pressure and pathogen spread.

Based on sticky trap catches there were little differences among the treatment categories with respect to natural enemy abundance in vineyards. We found similarly high catches of several natural enemy taxa, especially spiders, at all three types of sites (Fig. 2D). Tap sampling results showed a slightly different pattern. Generalist natural enemies were more common in untreated sites than mixed or consistently treated sites (Fig. 3A). However, this result does not appear to stem from disruption of natural enemy activity at treated sites. Rather, the abundance of all non-predatory arthropods (including several pest species such as grape leafhopper) were up to 7-fold higher on average at untreated sites compared to treated sites (Fig. 3B). Collectively these two sources of data indicate that imidacloprid applications do not dramatically upset natural enemy activity in vineyards. Rather, if anything, natural enemy populations may track pest populations, which are strongly affected by whether fields were treated with imidacloprid.

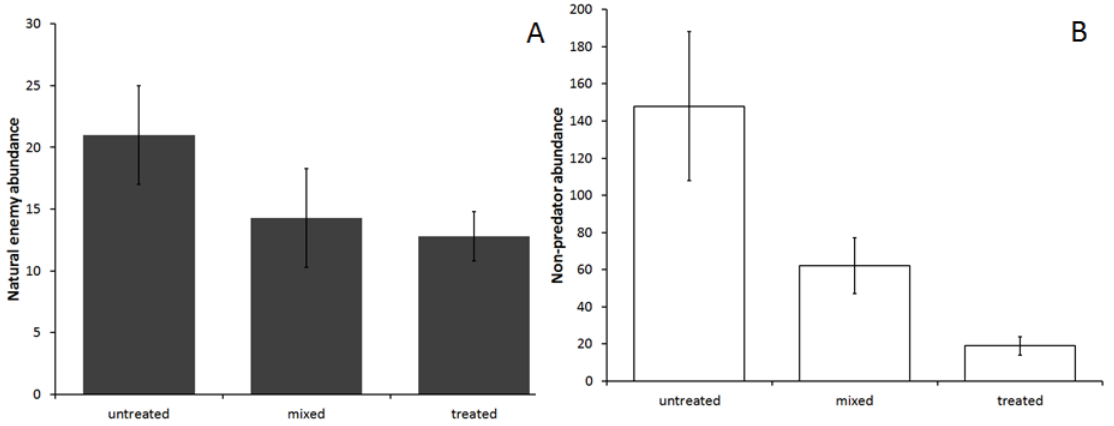

Fig. 3. A) Mean natural enemy abundance, and B) mean non-predatory arthropod abundance (+/- SE) based on tap sampling in Temecula Valley vineyards in 2012.

CONCLUSIONS

For many Southern California grape growers vector control is seen as a critical component to managing Pierce's disease within vineyards. Yet epidemiological theory suggests that disease management via vector control is only practical for pathosystems in which vectors are of limited efficiency. In such situations, as seems to be the case for *H. vitripennis*, the expectation is that reducing vector populations should limit the potential for pathogen spread.

Broadly, the work presented here suggests that within-vineyard chemical control

has the potential to reduce vector pressure and curb pathogen spread. Moreover, at least in this system, there do not appear to be strong non-target effects of the preferred systemic insecticide on natural enemies that would contribute to secondary pest outbreaks. However, three aspects of these results are worth exploring further. First, the observed differences in prevalence among years appear to be based on chemical control strategies, with untreated vineyards having the highest average prevalence, but whether those differences are due to recent management, historical artifacts, or differences in vector pressure at individual vineyards remains unclear. Ultimately analyses that grapple with year-to-year changes in prevalence are needed (i.e. disease incidence), which are ongoing. Second, it is interesting to note that there were little to no differences in Pierce's prevalence between the intermittently and consistently treated sites – especially in 2010 and 2011 surveys (MP Daugherty, unpublished data). This suggests that it may not be necessary to treat vineyards every year to effectively manage sharpshooter populations. Rather, in systems such as this one where there is substantial interannual variability in vector populations, targeting only the "outlier" years may be sufficient; assuming such years can be identified prior to treatment decisions. Finally, it is worth noting that such modest levels of observed disease are likely attributable to very low vector populations that exist currently relative to conditions during the peak of the *H. vitripennis* outbreak in the Temecula Valley region 15 years ago. The apparently slight differences in disease incidence among treatment categories might be expected to be substantially greater should the effective areawide control of sharpshooters be discontinued.

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